

1. A method of probabilistically classifying a sample of tissue of a mammalian anatomical structure, tissues of which may have various morphological and biochemical states and are classifiable in accordance therewith, comprising:

illuminating the tissue sample with first wavelength electromagnetic radiation  
5                   selected to stimulate in the tissues of the mammalian anatomical structure a  
                    fluorescence having spectral characteristics distinguishing between a first  
                    plurality of classifications therefor;  
                    acquiring first fluorescence intensity spectrum sample data for the tissue sample  
                    from the first wavelength illuminating step;  
10                   obtaining a first quantity from first fluorescence intensity spectral calibration data,  
                    the first calibration data being from a first calibration set comprising tissues  
                    in each one of the first plurality of classifications of a statistically significant  
                    set of tissues of the mammalian anatomical structures illuminated with the  
                    first wavelength electromagnetic radiation, and the first quantity accounting  
15                   for a significant amount of variation in the first calibration data and showing  
                    statistically significant differences between the first calibration set tissues in  
                    the first plurality of classifications;  
                    obtaining first probability distributions of the first calibration data as modified by the  
                    first quantity for each one of the first plurality of classifications; and  
20                   calculating from the first probability distributions and from the first sample data as  
                    modified by the first quantity a probability that the tissue sample belongs in  
                    one of the first plurality of classifications.

2. A method as in claim 1 wherein:

the first quantity obtaining step comprises obtaining a reduced principal component  
25                   matrix from application of a principal component analysis and a student's t-  
                    test;  
                    the probability distribution obtaining step comprises obtaining conditional  
                    probabilities from a model of probability distributions using a Gamma  
                    function; and

the probability calculating step comprises applying logistic discrimination using the conditional probabilities, the reduced component matrix, and the first sample data.

3. A method as in claim 1 further comprising:

5 illuminating the tissue sample with second wavelength electromagnetic radiation selected to stimulate in the tissues of the mammalian anatomical structure a fluorescence having spectral characteristics distinguishing between a second plurality of classifications therefor;

acquiring second fluorescence intensity spectrum sample data for the tissue sample  
10 from the second wavelength illuminating step;

obtaining a second quantity from second fluorescence intensity spectral calibration data, the second calibration data being from a second calibration set comprising tissues in each one of the second plurality of classifications of a statistically significant set of tissues of the mammalian anatomical structures  
15 illuminated with the second wavelength electromagnetic radiation, and the second quantity accounting for a significant amount of variation in the second calibration data and showing statistically significant differences between the second calibration set tissues in the second plurality of classifications;

obtaining second probability distributions of the second calibration data as modified  
20 by the second quantity for each one of the second plurality of classifications; and

calculating from the second probability distributions and from the second sample data as modified by the second quantity a probability that the tissue sample belongs in one of the second plurality of classifications, the second plurality  
25 of classifications being a refinement of one of the first plurality of classifications.

4. A method as in claim 3, wherein:

the first plurality of classifications is SIL and normal squamous, and the first wavelength is selected from 337 nm and 460 nm; and

the second plurality of classifications is high grade SIL and low grade SIL, and the second wavelength is 460 nm.

5. A method as in claim 3, wherein:

the first plurality of classifications is SIL and normal columnar plus inflammation,  
5 and the first wavelength is 380 nm; and  
the second plurality of classifications is high grade SIL and low grade SIL, and the second wavelength is 460 nm.

6. A method as in claim 1 further comprising:

illuminating the tissue sample with second wavelength electromagnetic radiation  
10 selected to stimulate in the tissues of the mammalian anatomical structure a  
fluorescence having spectral characteristics distinguishing between the first  
plurality of classifications; and

acquiring second fluorescence intensity spectrum sample data for the tissue sample  
from the second wavelength illuminating step;

15 wherein in the first quantity obtaining step, the first calibration data further includes  
spectral data from the first calibration set illuminated with the second  
wavelength electromagnetic radiation; and

wherein in the probability calculating step, the second sample data is included with  
the first sample data prior to modification thereof by the first quantity.

20 7. A method as in claim 6 wherein the first and second wavelength electromagnetic  
radiation being further selected to stimulate in the tissues of the mammalian anatomical  
structure a fluorescence having spectral characteristics distinguishing between a second  
plurality of classifications therefor, further comprising:

obtaining a second quantity from second fluorescence intensity spectral calibration  
25 data, the second calibration data being from a second calibration set  
comprising tissues in each one of the second plurality of classifications of a  
statistically significant set of tissues of the mammalian anatomical structures  
illuminated with the first and second wavelength electromagnetic radiation,  
and the second quantity accounting for a significant amount of variation in

the second calibration data and showing statistically significant differences between the second calibration set tissues in the second plurality of classifications;

obtaining second probability distributions of the first calibration data as modified by the second quantity for each one of the second plurality of classifications; and calculating from the second probability distributions and from the first and second sample data as modified by the second quantity a probability that the tissue sample belongs in one of the second plurality of classifications, the second plurality of classifications being a refinement of one of the first plurality of classifications.

8. A method as in claim 7, wherein:

the first plurality of classifications is SIL and normal squamous;  
the second plurality of classifications is high grade SIL and low grade SIL;  
the first wavelength is selected from 337 nm, 380 nm, and 460 nm; and  
the second wavelength is selected from 337 nm, 380 nm, and 460 nm.

9. A method as in claim 7, wherein:

the first plurality of classifications is SIL and normal columnar;  
the second plurality of classifications is high grade SIL and low grade SIL;  
the first wavelength is selected from 337 nm, 380 nm, and 460 nm; and  
the second wavelength is selected from 337 nm, 380 nm, and 460 nm.

10. A method as in claim 1 comprising repeating the illuminating step, the acquiring step, the first quantity obtaining step, and the calculating step for a plurality of contiguous tissue samples of the mammalian anatomical structure.

11. A method as in claim 10 wherein a first measurement comprises the illuminating and acquiring steps, a second measurement comprises the repeated illuminating and acquiring steps, and the first and second measurements are done *in vivo* and in succession.

12. A method as in claim 10 wherein a first measurement comprises the illuminating and acquiring steps, a second measurement comprises the repeated illuminating and

acquiring steps, and the first and second measurements are done *in vivo* and contemporaneously.

13. A method as in claim 10 wherein the anatomical structure is a human cervix, a first measurement comprises the illuminating and acquiring steps, a second measurement  
5 comprises the repeated illuminating and acquiring steps, and the first and second measurements are done *in vivo* in the ectocervix.

14. A method as in claim 10 wherein the anatomical structure is a human cervix, a first measurement comprises the illuminating and acquiring steps, a second measurement comprises the repeated illuminating and acquiring steps, and the first and second  
10 measurements are done *in vivo* in the endocervix.

15. A method as in claim 10 wherein a first measurement comprises the illuminating and acquiring steps, a second measurement comprises the repeated illuminating and acquiring steps, and the first and second measurements are done *in vitro* and in succession.

16. A method as in claim 10 wherein a first measurement comprises the illuminating  
15 and acquiring steps, a second measurement comprises the repeated illuminating and acquiring steps, and the first and second measurements are done *in vitro* and contemporaneously.

17. A method as in claim 1 wherein the first wavelength electromagnetic radiation consists of a narrowband excitation pulse centered on the first wavelength, the first sample  
20 data comprises continuous fluorescence intensity data over a continuous range of emission wavelengths, and the first calibration data comprises continuous fluorescence intensity data over the continuous range of emission wavelengths.

18. The method of claim 17 wherein the first wavelength is within one of the ranges of 317-357 nm, 360-400 nm, and 440-480 nm.

25 19. A method as in claim 1 wherein the first wavelength electromagnetic radiation consists of a narrowband excitation pulse centered on the first wavelength, the first sample data comprises fluorescence intensity data for a plurality of discrete emission wavelengths,

and the first calibration data comprises fluorescence intensity data for the plurality of discrete emission wavelengths.

20. The method of claim 19 wherein:

the first wavelength is within one of the ranges of 317-357 nm, 360-400 nm, and  
5 440-480 nm, and

the plurality of discrete emission wavelengths are about 410 nm, about 460 nm,  
about 510 nm and about 580 nm for an illumination of about 337 nm; about  
460 nm, about 510 nm, about 580 nm, about 600 nm and about 640 nm for an  
illumination of about 380 nm; and about 510, about 580 nm, about 600 nm,  
10 about 620 nm, about 640 nm and about 660 nm for an illumination of about  
460 nm.

21. A method as in claim 1 wherein the first sample data comprises preprocessed spectral data and the first calibration data comprises preprocessed spectral data.

22. A method as in claim 1 further comprising applying acetic acid to the tissue  
15 sample prior to the illuminating step.

23. A method of probabilistically classifying a sample of tissue of a mammalian anatomical structure, tissues of which may have various morphological and biochemical states and are classifiable in accordance therewith, comprising:

illuminating the tissue sample with electromagnetic radiation of a wavelength  
20 selected to stimulate in tissues of the mammalian anatomical structure a  
fluorescence having spectral characteristics indicative of a first classification  
thereof;

detecting a first fluorescence intensity spectrum from the tissue sample resulting  
from the illuminating step;

25 illuminating plural tissue samples from a calibration set with electromagnetic  
radiation of the wavelength;

detecting a plurality of second fluorescence intensity spectra from the tissue samples  
of the calibration set resulting from the plural tissue sample illuminating step;

dimensionally reducing the plurality of second fluorescence intensity spectra into a set of components that account for most of the variance in the second fluorescence spectra;

generating a subset of the components that are useful in placing in the first classification tissue samples from the calibration set belonging in the first classification; and

calculating from the first fluorescence intensity spectrum and the components a first probability that the tissue sample belongs in the first classification.

24. A method as in claim 23 further comprising preprocessing the plurality of second fluorescence intensity spectra prior to the dimensionally reducing step to reduce inter-sample and intra-sample variation.

25. A method as in claim 23 further comprising:

applying acetic acid to the tissue sample prior to the tissue sample illuminating step; and

applying acetic acid to the plural tissue samples from a calibration set prior to the plural tissue samples illuminating step.

26. A method of probabilistically classifying a sample of tissue of a mammalian anatomical structure, tissues of which may have various morphological and biochemical states and are classifiable in accordance therewith, comprising:

illuminating the tissue sample with electromagnetic radiation of a first wavelength selected to stimulate in tissues of the mammalian anatomical structure a fluorescence having spectral characteristics indicative of a first classification thereof;

detecting a first fluorescence intensity spectrum from the tissue sample resulting from the first wavelength illuminating step; and

calculating a first probability that the tissue sample belongs in the first classification from a data set comprising the first fluorescence intensity spectrum.

27. The method of claim 26, wherein the first wavelength is within one of the ranges of 317-357 nm, 360-400 nm and 440-480 nm.

28. The method of claim 26 wherein the first fluorescence intensity spectrum comprises emission wavelengths of about 410 nm, about 460 nm, about 510 nm and about 580 nm when the first wavelength is about 337 nm; about 460 nm, about 510 nm, about 580 nm, about 600 nm and about 640 nm when the first wavelength is about 380 nm; and about  
5 510, about 580 nm, about 600 nm, about 620 nm, about 640 nm and about 660 nm when the first wavelength is about 460 nm.

29. A method as in claim 26, further comprising:  
illuminating the tissue sample with electromagnetic radiation of a second wavelength  
selected to stimulate in tissues of the mammalian anatomical structure a  
10 fluorescence having spectral characteristics indicative of a second  
classification thereof;  
detecting a second fluorescence intensity spectrum from the tissue sample resulting  
from the second wavelength illuminating step;  
calculating a second probability that the tissue sample belongs in the second  
15 classification from a data set comprising the second fluorescence intensity  
spectrum; and.  
classifying the tissue sample in the second classification if the first and second  
probabilities exceed respective thresholds.

30. A method as in claim 26, further comprising:  
20 illuminating the tissue sample with electromagnetic radiation of a second wavelength  
selected to stimulate in tissues of the mammalian anatomical structure a  
fluorescence having spectral characteristics indicative of a second  
classification thereof;  
detecting a second fluorescence intensity spectrum from the tissue sample resulting  
25 from the second wavelength illuminating step;  
calculating a second probability that the tissue sample belongs in the second  
classification from a data set comprising the second fluorescence intensity  
spectrum;.  
illuminating the tissue sample with electromagnetic radiation of a third wavelength  
30 selected to stimulate in tissues of the mammalian anatomical structure a



fluorescence having spectral characteristics indicative of a second classification thereof;

detecting a third fluorescence intensity spectrum from the tissue sample resulting from the third wavelength illuminating step;

5 calculating a third probability that the tissue sample belongs in the third classification from a data set comprising the third fluorescence intensity spectrum; and

classifying the tissue sample in the second classification if the third, first and second probabilities exceed respective thresholds.

10 31. A method as in claim 30, wherein:

the third classification is SIL as distinguished from normal squamous, and the wavelength selected to stimulate in tissues of the mammalian anatomical structure a fluorescence having spectral characteristics indicative of the third classification thereof is selected for cervical tissues from 337 nm and 460

15 nm;

the first classification is SIL as distinguished from normal columnar and inflammation, and the wavelength selected to stimulate in tissues of the mammalian anatomical structure a fluorescence having spectral characteristics indicative of the first classification thereof is 380 nm for

20 cervical tissues; and

the second classification is high grade SIL as distinguished from low grade SIL, and the wavelength selected to stimulate in tissues of the mammalian anatomical structure a fluorescence having spectral characteristics indicative of the first classification thereof is 460 nm for cervical tissues.

25 32. A method as in claim 26 further comprising:

illuminating the tissue sample with electromagnetic radiation of a second wavelength selected to stimulate in tissues of the mammalian anatomical structure a fluorescence having spectral characteristics indicative of a first classification thereof; and

detecting a second fluorescence intensity spectrum from the tissue sample resulting from the second wavelength illuminating step;  
wherein the calculating step comprises calculating a first probability that the tissue sample belongs in the first classification from a data set comprising the first and second  
5 fluorescence intensity spectrum.

33. A method as in claim 32 further comprising:  
illuminating the tissue sample with electromagnetic radiation of a third wavelength selected to stimulate in tissues of the mammalian anatomical structure a fluorescence having spectral characteristics indicative of a first classification  
10 thereof; and  
detecting a third fluorescence intensity spectrum from the tissue sample resulting from the second wavelength illuminating step;  
wherein the calculating step comprises calculating a first probability that the tissue sample belongs in the first classification from a data set comprising the first, second and third  
15 fluorescence intensity spectrum.

34. A method as in claim 33 wherein the electromagnetic radiation of the first, second and third wavelengths further is selected to stimulate in tissues of the mammalian anatomical structure a fluorescence having spectral characteristics indicative of a second classification thereof, further comprising calculating a second probability that the tissue  
20 sample belongs in the second classification from a data set comprising the first, second and third fluorescence intensity spectrum.

35. A method as in claim 34 wherein the electromagnetic radiation of the first, second and third wavelengths further is selected to stimulate in tissues of the mammalian anatomical structure a fluorescence having spectral characteristics indicative of a third  
25 classification thereof, further comprising calculating a third probability that the tissue sample belongs in the third classification from a data set comprising the first, second and third fluorescence intensity spectrum.

36. A method as in claim 35, wherein:  
the first wavelength is about 337 nm;

- the second wavelength is about 380 nm;
- the third wavelength is about 460 nm;
- the third classification is SIL as distinguished from normal squamous;
- the first classification is SIL as distinguished from normal columnar; and
- 5 the second classification is high grade SIL as distinguished from low grade SIL.

37. A method as in claim 26 wherein the calculating step comprises calculating a probability from the first fluorescence intensity spectrum that the tissue is SIL versus normal squamous.

38. A method as in claim 37 wherein the illuminating step comprises illuminating the  
10 tissue sample with electromagnetic radiation having a wavelength of about 337 nm.

39. A method as in claim 37 wherein the illuminating step comprises illuminating the tissue sample with electromagnetic radiation having a wavelength of about 460 nm.

40. A method as in claim 26 wherein the calculating step comprises calculating a probability from the first fluorescence intensity spectrum that the tissue is SIL versus normal  
15 columnar and inflammation.

41. A method as in claim 40 wherein the illuminating step comprises illuminating the tissue sample with electromagnetic radiation having a wavelength of about 380 nm.

42. A method as in claim 26 wherein the calculating step comprises calculating a probability from the first fluorescence intensity spectrum that the tissue is high grade SIL  
20 versus low grade SIL.

43. A method as in claim 42 wherein the illuminating step comprises illuminating the tissue with electromagnetic radiation having a wavelength of about 460 nm.

44. The method of claim 26 wherein the illuminating step is performed *in vivo*.

45. The method of claim 26 wherein the illuminating step is performed *in vitro*.

46. A method of assigning a probability that a tissue sample belongs to a particular tissue category, comprising:

providing a first tissue sample;

illuminating the first tissue sample with electromagnetic radiation having at least one wavelength known to excite tissue into producing a fluorescence intensity spectra containing information about whether tissue belongs to the particular tissue category;

detecting a fluorescence intensity spectra from the first tissue sample; and

calculating from the fluorescence intensity spectra from the first tissue sample a probability that the tissue sample belongs to the particular tissue category.

47. A method as in claim 46 wherein the calculating step comprises:

providing a statistically significant plurality of second tissue samples, at least some of which are tissues known to belong to the particular tissue category;

illuminating the second tissue samples with the electromagnetic radiation;

detecting a plurality of fluorescence intensity spectra from the second tissue samples, respectively;

calculating from the fluorescence intensity spectra from the second tissue samples a probability distribution for the second tissue samples belonging to the particular tissue category; and

calculating the probability that the tissue sample belongs to the particular tissue category using the fluorescence intensity spectra from the first tissue sample and the probability distribution for the second tissue samples.

48. A method as in claim 46 wherein the probability distribution calculating step comprises:

generating a set of first vectors that account for variation in the fluorescence intensity spectra from the second tissue samples; and

selecting from the first vectors a set of second vectors that are indicative of the particular tissue category, the second vectors containing first indicia of the probability distribution for the second tissue samples belonging to the particular tissue category.

49. A method as in claim 48 wherein:

the first vector generating step comprises principle component analysis;

the second vector generating step comprises a student's t-test; and

the step of calculating the probability that the tissue sample belongs to the particular  
5 tissue category using the fluorescence intensity spectra from the first tissue  
sample and the probability distribution for the second tissue samples  
comprises logistic discrimination.

50. A method as in claim 46 wherein:

the illuminating step comprises illuminating the tissue sample with electromagnetic  
10 radiation having at least a first wavelength known to excite tissue into  
producing a fluorescence intensity spectra containing information about  
whether tissue belongs to a first tissue category, and a second wavelength  
known to excite tissue into producing a fluorescence intensity spectra  
containing information about whether tissue belongs to a second tissue  
15 category that is a refinement of the first tissue category;

the detecting step comprises detecting first and second fluorescence intensity spectra  
from the illuminating step to obtain respective first and second spectral data;  
and

the calculating step comprises calculating from the first spectral data a first  
20 probability that the tissue sample belongs to the first tissue category,  
calculating from the second spectral data a second probability that the tissue  
sample belongs to the first tissue category, and assigning the tissue sample a  
probability of belonging to the second tissue category from the first and  
second probabilities.

51. A method as in claim 46 wherein:

the illuminating step comprises illuminating the tissue sample with electromagnetic  
radiation having a first wavelength of about 337 nm, a second wavelength of  
about 380 nm, and a third wavelength of about 460 nm; and

the detecting step comprises detecting first, second and third fluorescence intensity spectra from the illuminating step to obtain respective first, second and third spectral data; and

calculating from the first, second and third spectral data a probability that the tissue sample belongs to the particular tissue category.

52. An apparatus for probabilistically classifying a sample of tissue of a mammalian anatomical structure, tissues of which may have various morphological and biochemical states and are classifiable in accordance therewith, comprising:

a controllable illumination source for generating electromagnetic radiation of a wavelength selected to stimulate in the tissues of the mammalian anatomical structure a fluorescence having spectral characteristics distinguishing between a plurality of classifications therefor;

an optical system for illuminating the tissue sample with the electromagnetic radiation and acquiring fluorescence emissions from the tissue sample;

a detector for converting the fluorescence emissions from the tissue sample to intensity spectrum sample data;

a processor coupled to the controllable illumination source for control thereof and coupled to the detector for processing the sample data, the processor comprising:

means for storing a quantity obtained from fluorescence intensity spectral calibration data, the calibration data being from a calibration set comprising tissues in each one of the first plurality of classifications of a statistically significant set of tissues of the mammalian anatomical structures illuminated with the electromagnetic radiation, and the quantity accounting for a significant amount of variation in the calibration data and showing statistically significant differences between the calibration set tissues in the plurality of classifications;

means for storing probability distributions of the calibration data as modified by the first quantity for each one of the plurality of classifications; and

means for calculating from the probability distributions and from the sample data as modified by the quantity a probability that the tissue sample belongs in one of the first plurality of classifications.

5 53. A computer program product comprising a computer readable medium having program logic recorded thereon for probabilistically classifying a sample of tissue of a mammalian anatomical structure, tissues of which may have various morphological and biochemical states and are classifiable in accordance therewith, comprising:

10 means for controlling illumination of the tissue sample with electromagnetic radiation of a wavelength selected to stimulate in the tissues of the mammalian anatomical structure a fluorescence having spectral characteristics distinguishing between a plurality of classifications therefor;

means for controlling acquisition of fluorescence intensity spectrum sample data for the tissue sample;

15 a quantity obtained from fluorescence intensity spectral calibration data, the calibration data being from a calibration set comprising tissues in each one of the plurality of classifications of a statistically significant set of tissues of the mammalian anatomical structures illuminated with the electromagnetic radiation, and the quantity accounting for a significant amount of variation in the calibration data and showing statistically significant differences between  
20 the calibration set tissues in the plurality of classifications;

first probability distributions of the calibration data as modified by the first quantity for each one of the plurality of classifications; and

25 means for calculating from the probability distributions and from the sample data as modified by the quantity a probability that the tissue sample belongs in one of the plurality of classifications.